Using SpatialDE to Characterize Spatiotemporal Changes in Mitochondrial Morphology

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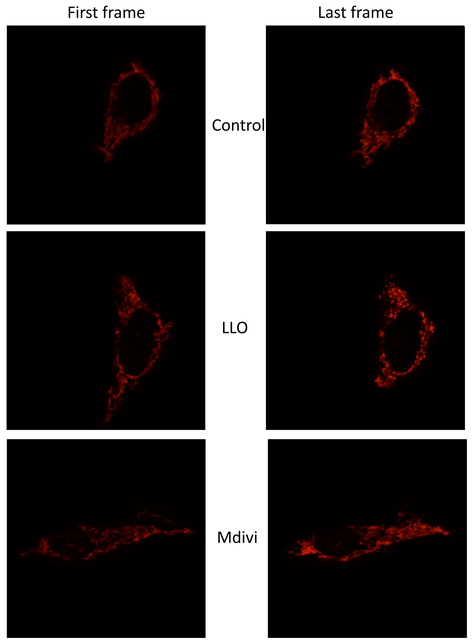
Introduction

Subcellular structures are highly susceptible to pathogenic hazards, and recent advances in fluorescence microscopy have been extremely useful in visualizing the morphological changes that occur in response to these stimuli. Understanding exactly how intracellular bacterial pathogens interact with the target organelle's orientation in space and time has crucial implications for the potential of targeted treatments. However, visualization alone is not an effective strategy due to the subjectivity involved and a lack of efficiency at high throughput. Currently, there are methods for modeling and quantifying variations in solid structures, such as cells, but these methods are lacking for more diffuse subcellular components, such as mitochondria. Work is being done to consider Gaussian Mixture Models as a viable solution by viewing mitochondria as social networks, but there are difficulties with this method. It is unclear how to choose the number of nodes, as well as the fact that there is no sense of continuity between frames in time series data.

The current project explores the feasibility of SpatialDE as an alternative solution. Published in *Nature* in 2018 along with full code online, SpatialDE is a method for identifying genes that are spatially variable in expression; that is, their orientation in space is a key determinant of their function and behavior. We theorize that a novel application of this method can use bioimaging data to quantify the social networks visually seen between organelles such as mitochondria.

Data

Time series footage was taken of HeLa cells transfected with DsRed2 to tag mitochondria. Two stimuli and a control were examined.

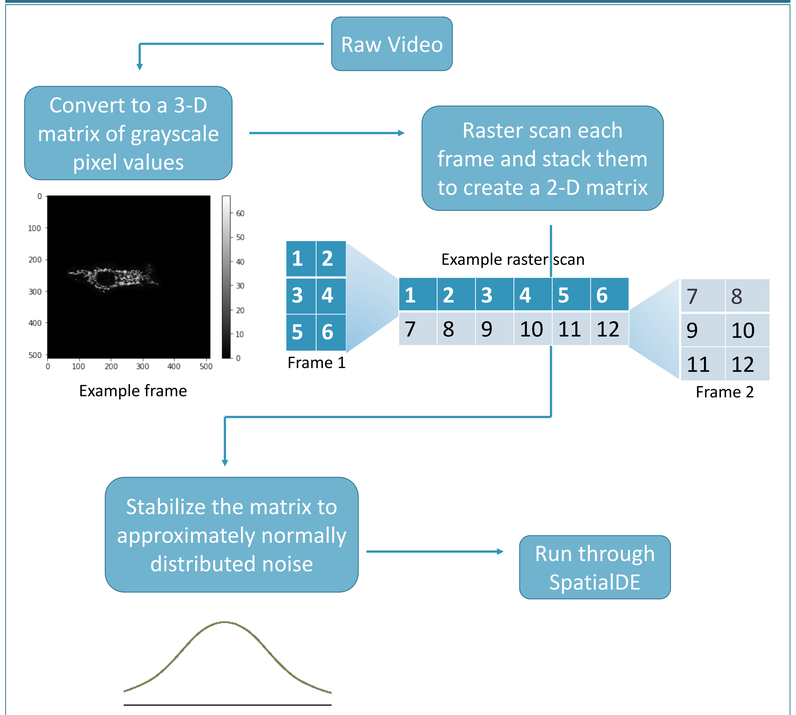


Control
- Normal, wild-type mitochondria
- Establishes baseline movement in space and time

LLO (Listeriolysin O)
- A hemolysin produced by the bacteria that causes listeriosis
- Causes fragmentation in the mitochondria

Mdivi
- A mitochondrial division inhibitor
- Brings the proteins together in fusion

Methods



Results

The output given by SpatialDE is a data frame with 18 columns of various information for each pixel, thus 262,144 rows for a 512x512 frame video. Some of the most relevant columns are:

- FSV—The fraction of variance explained by spatial variation
- LLR—Log-likelihood ratio, the ratio of an alternative model, H_1, which includes spatial and non-spatial components, to the null model, H_0, which does not include the spatial variance component
- pval—The P-value for spatial differential expression
- qval—Significance after correcting for multiple testing

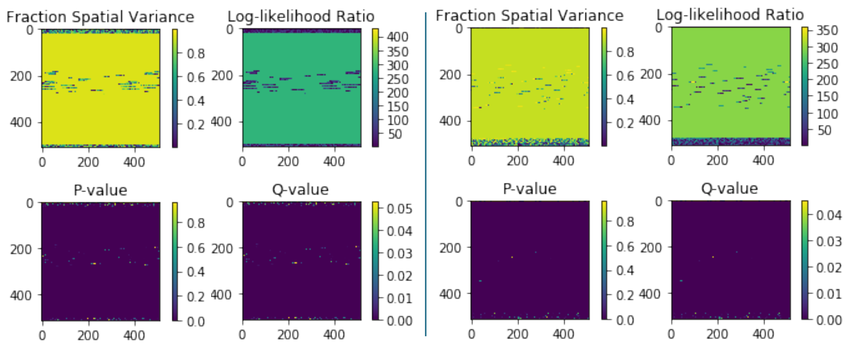


Figure 1. The results of a sample control (left) and LLO (right) cell transformed back into an "image," so each coordinate corresponds to the pixel at that location throughout the time series.

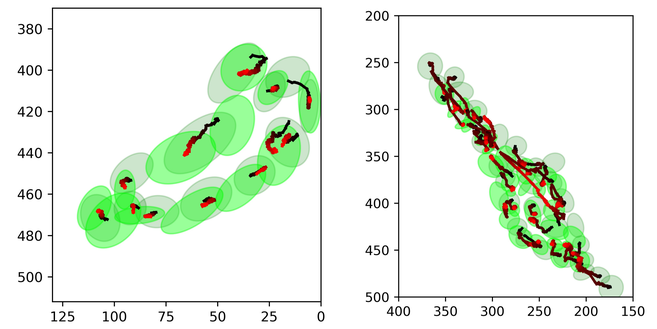


Figure 2. Gaussian Mixture Model representation of the mitochondria in a control cell (left) and an LLO cell (right). The darker green ovals represent the nodes of the starting components, and the lighter green represents the same for the ending components. The lines represent the component trajectories over time, using their means, with black indicating the starting frame and red indicating the last frame.

Discussion

The results of SpatialDE did not reveal any meaningful patterns to aid in drawing comparisons between cells of the same treatment or among the different treatments. There is a definite non-random pattern among the output, but it is unclear why there is not a clearly-defined cell shape. It would be expected that all the cells of one category would share similar results, but this does not hold true. Therefore, it is impossible to draw conclusions between a control cell and an LLO cell, because the differences cannot be attributed solely to the treatment. The Gaussian Mixture Model exemplifies the type of results desired. There is a clear distinction between the control cell, whose components show little movement during the time period, and the LLO cell, whose components show a distinct trajectory that mimics the fragmentation that is clearly seen in the imaging data. Therefore, compared to the work that has already been accomplished by the Gaussian Mixture Model method, SpatialDE appears to be an inferior method of measuring covariance between the components, mostly due to the format of the results.

Conclusions and Future Work

Currently, SpatialDE does not seem like a practical solution for developing a spatiotemporal model for diffuse organelles. We have contacted the developers to see if they have any insight into drawing conclusions from our results, so we will continue to pursue this alternative in the future. However, currently Gaussian Mixture Models continue to be the best option. Moving forward, the next step is to implement a temporal component, so the model is more accurate and efficient in describing how the social networks change over time, as opposed to viewing each frame independently of the next. Additionally, a single uniform component is necessary to be able to compare its output to that of different methods, such as SpatialDE. Without this, it is impossible to directly compare the two. Once a single spatiotemporal model is completely implemented, we can better understand the mechanisms by which bacterial virulence factors transform organelle structure in host cells and thus affect biological functions that are necessary for life.

Acknowledgements

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